REMARKS:

In this response, the currently pending claims are claims 10-13, 18-20 and 22-26. No new matter has been introduced into the application. Support for new claims 22-26 may be found *inter alia* in the application and in previous claims 14-17. Support for the addition of the term "peroxidases" in claim 20 is supported in the application, *inter alia*, on page 51 under Section 5.15.3 entitled "The Reaction" in the first sentence: "The cross-link reaction can utilize any chemical reaction or physical known in the art that specifically introduces dityrosine cross-links, such as peroxidase catalysed cross-linking, or photodynamically in the presence or absence of sensitizes (see Section II)."

Claims 10-13, 18-20 and 22 remain pending and under examination in this application. Withdrawn claims 1-9, 14-17 and 21 have been canceled without prejudice to applicants' right to pursue the subject matter of these claims in another application. Claims 10-13, 18-20 and 22 have been amended herein. Claims 23-26 have been introduced herein to more clearly claim the present invention. These amendments raise no issue of new matter.

Objections to Claims Under 37 C.F.R. § 1.75(c)

The Examiner objected to claims 14 and 17 as being of improper dependent format for allegedly failing to further limit the subject matter of a previous claim. These claims were also objected to for depending upon a non-elected claim.

In reply, applicants have canceled claims 14 and 17 without prejudice. Accordingly, applicants request the Examiner to reconsider and withdraw this ground of objection.

Rejections Under 35 U.S.C. § 112, first paragaph

Claims 10-17 were rejected under 35 U.S.C. §112, first paragraph, as allegedly not enabled by the specification. The Examiner stated that the specification is enabling for "an

isolated, stabilized protein, comprising isolating a polypeptide, selecting one or more tyrosine residue pairs in a polypeptide chain, cross-linking the tyrosine residue pair(s) under defined conditions...." However, the Examiner stated that the specification is not enabling for "any isolated protein comprising a di-tyrosine cross-link by genetic engineering." (See, p. 3, Office Action).

In reply, applicants have amended claim 10 to more clearly recite the claimed invention. The claim no longer includes the language "cross-link by genetic engineering." Claim 10 is now directed to an isolated protein comprising at least one di-tyrosine cross-link wherein at least one tyrosine of the di-tyrosine cross-link is from a point mutation to tyrosine, and wherein the protein retains at least one function displayed by the protein in the absence of di-tyrosine cross-linking. The Examiner admitted that genetic modification of the protein structure to introduce a substitution or addition of an amino acid tyrosine "is well known." Thus, applicants maintain that the claims are fully enabled by the specification.

Accordingly, applicants respectfully requests reconsideration and withdrawal of this ground of rejection.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 10-17 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. In particular, the Examiner stated that the claims are indefinite because a di-tyrosine cross-link cannot be introduced by genetic engineering.

In reply, applicants traverse the rejection, but in the interest of accelerated prosecution, have amended claim 10, and have canceled claims 14-17 without prejudice. Claim 10 has been amended to more particularly point out the presently claimed invention. In particular, the language "introduced by genetic engineering" has been deleted from the claim. In view of the amendments, applicant requests the Examiner to reconsider and withdraw this ground of rejection.

Rejections Under 35 U.S.C. §102(b)

Aeschbach et al.

The Examiner rejected claims 10, 12, 17-19 & 22 under 35 U.S.C. §102(b) as being anticipated by Aeschbach et al. (1976), BBA 439, 292-302 (AA – IDS). The Examiner stated that Aeschbach et al. teach a method for formation of di-tyrosine cross-links in proteins, the hormone insulin for example, by oxidation using hydrogen peroxide. The Examiner takes the position that the reference teaches all of the elements of the claims.

In reply, applicants respectfully traverse the rejection and assert that the claims as presented herein are not anticipated by the Aeschbach et al. reference. Claim 10 is directed to an isolated protein comprising at least one di-tyrosine cross-link, wherein at least one tyrosine of the di-tyrosine cross-link originates from a point mutation to tyrosine, and wherein the protein retains at least one function displayed by the protein in the absence of di-tyrosine cross-linking.

Aeschbach et al. disclose dityrosine (DT) bond formation in a variety of proteins, including insulin, trypsin, chymotrypsin, pepsin, and papain, that "residual" protein function (chymotrypsin & trypsin retain 50% and 35% of catalytic function, respectively: page 298 under "Influence of Oxidation Upon the Activity of Different Enzymes"). The paper discloses DT bond formation to be intra- and intermolecular (figure 5: gel filtration assay of oxidized insulin, and page 300, 2nd paragraph under "Properties of Oxidized Proteins").

However, Aeschbach et al. do not disclose a method of DT bonding that involves any of: analyzing protein structure, selecting residues, introducing point mutation to tyrosine (resulting in one or more tyrosine pairs in close proximity that can form DT bonds) or phenylalanine (where tyrosine residues are identified that would form undesirable DT bonds), and cross-linking under optimized conditions to minimize oxidative damage to the protein. Thus, Aeschbach et al. do not disclose "wherein at least one tyrosine of the di-tyrosine cross-link originates from a point mutation to tyrosine" as required by claim 10.

Therefore, applicants assert that the claims are not anticipated by the Aeschbach et al. paper and request the Examiner to reconsider and withdraw this ground of rejection.

Brown et al.

The Examiner rejected claims 10-13, 17-20 & 22 under 35 U.S.C. §102(b) as being anticipated by Brown et al. [*Biochemistry*, 1998, 37: 4397-4406, AD – IDS]. The Examiner took the position that Brown et al. teach protein-protein cross linking to be mediated by Ni(II) complex of the tripeptide gly-gly-his fusion protein, target being tyrosine, in the presence of oxidants such as oxone and monoperoxyphalic acid (MMPP) and a method of making the cross-linked peptide.

In reply, applicants traverse the rejection and assert that the pending claims are not anticipated by the Brown et al. reference. Brown et al. disclose the formation of GGH-ecotin homodimer (pp.: 4399-4401 under "GGH-Fusion Protein Supports Cross-Linking" and " The Cross-Linking Reaction Occurs within the Dimeric Ecotin Complex"; figures 1 & 2), and the formation of an ecotin-trypsin heterodimer (p. 4401 under "GGH-Ni(II)-Ecotin Can Be Cross-Linked to Its Protease Targets"; figure 3), both only in the presence of Ni(II) and MMPP (oxidizing conditions). The paper discloses that complex formation is demonstrated to be due to DT bond formation (pp. 4401-4402 under "The Target of the Cross-Linking Reaction Is Tyrosines"; figure 4). Furthermore, the paper discloses the enhanced formation of DT bonded dimers of an ecotin point mutant containing the amino acid substitution Asp137Tyr (pp. 4402-4403 under "Rational Protein Engineering Can Dramatically Increase the Efficiency of the Cross-Linking Reaction"; figures 5 & 6). Asp137Tyr mutant forms DT bonded dimers with an efficiency of approximately 60%, by comparison to wild-type that forms DT bonded dimers with an efficiency of approximately 15-20% (p. 4402, figure 6).

However, the paper does not disclose a DT bonded (or containing) protein that retains function. In particular, in Brown et al., the crosslinking reaction conditions are not optimized to minimize oxidative damage.

The authors do not describe cross-linked ecotin dimer binding to, and/or inhibiting a serine protease. Ecotin is a serine protease inhibitor that binds to the enzyme as a homodimer to inhibit its function. In fact, Brown et al. disclose that "interestingly, no ecotin2-trypsin2 complex was observed" (p. 4401 under "GGH-Ni(II)-Ecotin Can Be Cross-Linked to Its

Protease Targets", first paragraph), which can be explained by cross-linked ecotin's destroyed ability to bind to trypsin.

Claim 10 requires that "the protein retains at least one function displayed by the protein in the absence of di-tyrosine cross-linking." Nowhere does Brown et al. disclose that by introducing point mutations into a protein to increase the crosslink efficiency could a protein be engineered that retains such function. Throughout the Brown et al. paper, the utility of DT cross-linking is solely focused on detecting protein-protein interactions in solution/lysate due to the specificity of the reaction. Increased crosslink efficiency is only specified as "beneficial toward efforts to identify protein-protein interactions in large complexes." Therefore, the above-recited element of claim 10 is not disclosed by Brown et al. Accordingly, applicants request that the Examiner reconsider and withdraw this ground of rejection.

Rejected Under Doctrine of Obviousness-type Double Patenting

Claims 9-40 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 16-39 of co-pending patent application U.S. Serial No. 09/214,645.

In reply, applicants request that the Examiner hold this rejection in abeyance until there is an indication of allowable subject matter.

CONCLUSION:

Applicants respectfully submit that the application and claims are now in condition for allowance. If the Examiner believes that further discussion would be helpful, he is respectfully requested to telephone the undersigned attorney at (212) 937-7233 and is assured of full cooperation to advance the application to allowance.

Should an extension of time be required to make the filing of this response timely in the U.S. Patent and Trademark Office, the Commissioner is respectfully requested to grant any such Petition for an Extension of Time that is required for the timely filing of this response and is hereby authorized to charge any fee(s) for such a Petition for an Extension of Time that may be necessary in this application to Deposit Account No. 08-0219, Order No. 289550-122US1.

In addition, should any fees, or additional fees, be deemed to be properly assessable during the pendancy of this application, or for the timely consideration of this response, the Commissioner is hereby authorized to charge any such additional fee(s), or to credit any overpayment, to Deposit Account No. 08-0219, Order No.289550-122US1).

Respectfully submitted,

HALE AND DORR LLP

Date: 2/04/04

By: Jane M Love

Jane M. Love, Ph.D.
Registration No. 42,812

Correspondence Address:
HALE AND DORR LLP
300 Park Avenue
New York, New York 10022
Telephone: (212) 937-7200
Facsimile: (212) 937-7300